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NEMATODES IN SEWAGE AND SEWAGE TREATMENT FACILITIES

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FINAL PROGRESS REPORT

NEMATODES IN SEWAGE AND SEWAGE TREATMENT FACILITIES

FEDERAL WATER POLLUTION CONTROL ADMINISTRATION

Research Grant WP-00055

James V. Walters
Associate Professor of Civil Engineering
Principal Investigator

UNIVERSITY OF ALABAMA
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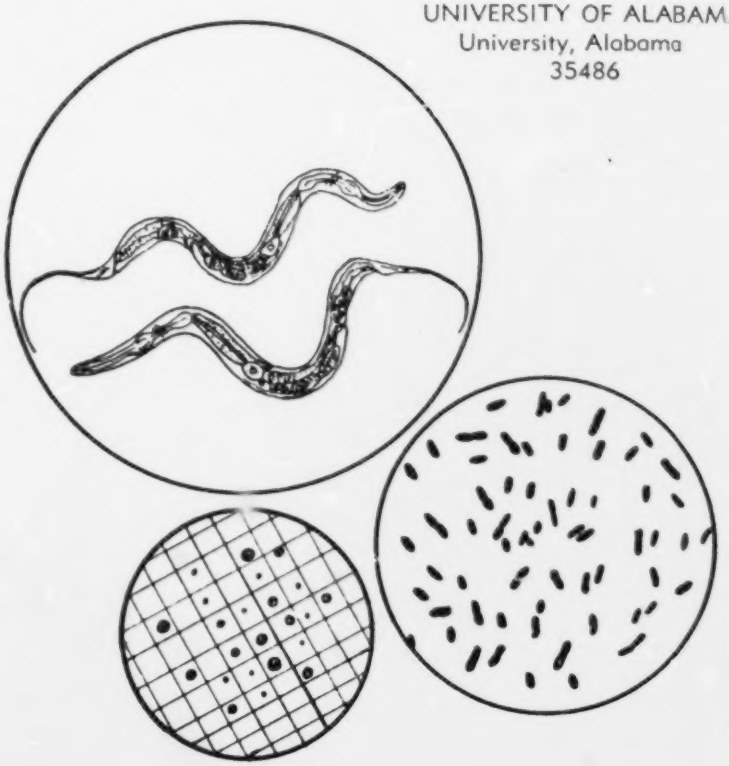
35486



Report Prepared:
January 1968

Grant Period:
June 1, 1963 thru
November 30, 1967

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The overall objective of the research summarized here has been the evaluation of the possibility and probability of nematodes functioning as vectors of water-borne diseases. The system of events by which such disease transmission might occur is somewhat complex and therefore will be described in unit steps.

Both nematodes and pathogenic bacteria are found in raw sewages. Sewage treatment plants provide conditions of quiescent flow and detention during which nematodes might graze upon bacteria. Some nematodes escape the treatment plant with the effluent water and thus may possibly pass downstream to the intake of a water treatment plant.

Coagulation and sedimentation do not trap all nematodes which enter the raw water intake of a water treatment plant. Chlorine, in the concentrations used for water supply disinfection, does not kill all the nemas which escape the sedimentation basin and some of the living worms which reach the rapid sand filters can wriggle thru the entire thickness of the filter medium. Such nematodes could be capsules of bacteria for the unwary drinker. If the undesirable bacteria could be released in the digestive tract of the drinker, then the nematode should have functioned as a vector.

Evaluation of the entire vector system was attempted thru the demonstration of the possibility of each step not already demonstrated by others and thru the study of the corresponding probability. Unit objectives of the study concerned these individual steps.

We wished to discover which nematodes were normally found in sewages and in what concentrations of population they might be expected. To obtain such population information we investigated the sewages influent to at least two plants of each of the three types studied and also examined influents to sewage lagoons. Preliminary examinations of sewages influent to and effluent from sewage oxidation lagoons indicated probably the more productive utilization of resources upon continued intensive investigation of traditional sewage treatment systems. Similarly it was found that specific identification of each isolated nematode was not worth the cost of taking routine identifications past the genera.

A second unit objective was the evaluation of the effects of the various sewage treatment unit processes upon the nematode populations. Satisfactory accomplishment of the two aims already specifically mentioned required the design and testing of a methodology for sampling and counting sewage-borne nematodes and for evaluating the effects of the process entities upon the enumerated populations. The methodology was used extensively at one of each of the three major types of treatment plants, primary, trickling filter, and activated sludge. Similar studies at other plants were performed to demonstrate the representative nature of the results of the intensive studies at the three plants mentioned.

The third group of questions to be answered during the study centered upon the nature and numbers of bacteria which nematodes might ingest while passing thru a sewage treatment plant. It would have been desirable to investigate the number of each bacterial pathogenic species which nematodes ingested in the studied plants, but the magnitude and logistics of the bacterial diagnostic procedures involved lead us to choose more practical goals. This practicality lead us to forego

diagnostic examination of nemal gut contents for the genera Mycobacterium and concentrate our abilities upon examinations for bacteria characteristic of fecal pollution and gastroenteritic epidemics.

All of the chosen goals mentioned specifically above have been satisfactorily accomplished. The methodologies were established, representative populations were mensurated, worm removal efficiencies of the unit processes were evaluated, and it was demonstrated that nematodes ingest bacteria while in sewage treatment plants. Moreover, pathogenic bacteria were found in the digestive tracts of nematodes taken from sewage treatment plant sampling points. The details of all these findings have been reported to the profession in one or more of the publications and presentations which comprise the list of references below.

An objective suggested by results of the intranematodal bacteria studies was to find an explanation for why no Gram-positive bacteria were found in the gut of nematodes from sewage treatment plants when such bacteria are abundantly present in the sewages. Rapid lysis of the bacteria after their possible ingestion by the nematodes was one thesis proposed to explain the observed absence of this group from the bacteria isolated from the nematodes. Immediate bacterial analyses of worm gut contents in the field were suggested as a possible means of evaluating the rapid lysis thesis. The burdensome logistics of such a procedure lead to the improvization of an alternate laboratory procedure. Tagged species of this bacterial group were grown in culture dishes and nematodes were observed as they grazed upon the organisms. Two explanative mechanisms appeared to be active. Those bacteria which were ingested were lysed in less time than was required for sampling, transportation, and analysis of sewage used in the earlier studies. Secondly, some of the bacteria appeared to be somewhat toxic to the worms.

After adequate demonstration of the ingestion of pathogenic bacteria by nematodes found in sewage treatment plants, it was necessary to learn whether nemas could excrete or otherwise release viable bacteria. (The ability of nematodes to pass thru water treatment plants in viable condition had already been demonstrated in studies other than those herein. reported.) Nematodes were found able to excrete or release viable bacteria while in a food-rich environment but were not found to do so during intervening periods while suspended in only buffered dilution water. These two differing environments are in some ways analogous to the food-rich sewage of a treatment plant and the food-poor water of a receiving surface-water body.

In order to simulate the conditions nematodes might encounter in the digestive tracts of humans, a series of experiments were performed in which nematodes were exposed to enzyme systems analogous to those of human digestion. The anticipated possible lysis of the nematodes did not occur but it appeared that the worms suffered coma and tetany in the high and low pH enzymes respectively. In other studies it was shown that even after the death of nematodes, viable bacteria can escape the oral and anal openings as the apparent result of Brownian movement. Rupture of the pelicle of dead females allows the escape of the viable bacteria present when a newly-hatched larva breaks out of the mother's body.

An intermediate objective of the studies described immediately above was the axenic culture of nematodes for such laboratory studies. Axenic culture was finally achieved after the attempts with several unsatisfactory methods. The successful method involves the collection and chlorine sterilization of ova and the subsequent placing of the eggs in a sterile medium for hatching. No medium was found with which the nematodes could

be maintained axenically thru complete life and reproduction cycles, but it was found that worms from such axenic cultures could be planted on previously axenic cultures of a bacterial species isolated from sewage and thereon could complete full life and reproduction cycles.

The general goal of this research as well as the specific goals mentioned above were accomplished as described. In addition to these, at various times during the period of the grant it appeared desirable to pursue four other objectives. With the availability of the techniques developed it would be useful to determine the normal bacterial flora of nematodes in habitats other than sewage. It would also be helpful to perform population evaluations upstream and downstream from sewage treatment facilities to determine the effect of the general nematodal intestinal bacterial fauna. A further extension of such studies would logically involve similar investigations in the various units of a downstream water supply system. These three objectives were found to be broader in scope than the resources available for this research could support. Fortunately much of this work is now being pursued by investigators at other institutions so that the information may be obtainable soon for others interested in these problems.

The fourth optional goal involved the evaluation of iodine for possible use in obtaining axenic cultures of nematodes. The successful method finally developed for such culturing made this anticipated work unnecessary.

As described above, almost all of the unit goals have been achieved during the satisfactory pursuit of the major aims of the research. Detailed reporting of most of the methods and results summarized here have been recorded in the presentations and publications which are entries of

the reference list below. A few experiments remain to be published. When they have been, copies will be forwarded for the use of the FWPCA.

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